Fla⁻ $H_1^{non\,b}$ segregants. 1256

DATE: APR 2 8 1955 REF:

1 2 3 4 5 6 7 8 9 10

1 1252A2 } a-x b
2 " }
3 1252B2 } trails? w/ b serum.

Test by two agglutinins. Isolate $Fla^- H_1^x$

B) x— FA10 (H_1^b) + b serum ⟶ ? ⟶ $Fla^+ H_1^b$

A) x— FA93 H_1^a + a serum. If x = a, no swarms. If x = b, H_1^x = b.

Results:

1 - Not pure motile. Cover to DCC
2 - Pure motile. 3 { B no swarm
3 - " " A swarm ⟶

20

30

40

50

DATE: MAY 3 1955 REF:

A. Staining in situ W1177.

a. in mixture c .005% TZ under oil — No stg. overnight Some inhibition ?

b. In methocel 4000 2% (in Penassay) + c .1% TZ, isolated colonies only (resistant ?) — stained only in center.

? are these conditions too aerobic ? Otherwise potentiate toxicity of TZ

Should reisolate colonies; compare growth c .5 TZ under oil.

B. Chains from isolated cells. (One c. 4-6 days thereafter)

A4

1. In scattered region many chains showed interstitial lethals. (effect of cold ?)

2. Few Z granules now seen.

3. Isolates ruined by spread of growth under them (film of moisture from drops !) A - B - C

D 2 is single

D 4 c 20 cells, Z term.

5 n.g.

6 n.g.

7 - 10 n.g.

E 1. single, Z term.

2 - 5 n.g.

6 no Z

7 no Z

8 no Z

9 n.g.

Z chains etc. 1258

Motility in Methocel solns.

DATE: MAY 4 1955 REF:

A. attempts at clearing cells in pectin, according in relation to 28μ MMM beads, in Methocel 4000 cps 2% is necessary.

General conclusions as stated ~~attached~~ - see 1258 protocol.

Some Z+ chains c. 100 cells terminal Z cell still intact. ~~Intercalated~~ detached noted probably more frequent after refrigeration, but this is not settled by direct observation.

B. 5/6/55. Set up to repeat 1254. Began OK but slow to divide at RT (though warm) & later lost c.g.

C. " Methocel 400 cps seems to show up motile cells (1257 A1+). Put trials as selector for E cells. But must used 4000 in trial.

D. See 5/8/55. E - a slump: did not completely inhibit motile cells upstairs.

1) Possible use of Scotchbrite MMM beads as reference markers - time is a slow drift; May be better to use 3% Methocel 4000 rather than 2%

2) Wasted expt. in completing E, Z chains

3) Pulmi expt. in screening E, S cells by reservoir medium strikingly successful. See ff.

4) But most time last week or 10 days was wasted exc. to improve general impression of technique.

5/6/00 1258

Lab plans: what to doS? Things are a mess.

1. Currently enmeshed in hhe fate of Z granules. Can these really givr any important information? By following a granule during the growth of a single cell, one might get a clue as to whether groth is interstitial or bipolar (jn a few cases). To distinguih, one might have to show increasing separation between two granules, before fission in a single cell and thismay be difficult.
It is already clear that 1) terminal granules usually remain terminal, and that this is the most common type, already suggesting a polarity in the cell. Occasionally, bi-antipolar cells are seen (more commonly that bi-synpolar), suggesting that the two poles share something distinct from the fissile center. However, the basic interest in the Z granule for the current problem is the possible correlation with E, and this, if anything is what should be pursued for now. Later it may be conveneint to try to bepeat experiments with a polar-flagellated organism.
Another sideline is to use the shaihs in stiff medium to study other problems, chiefly lethalitym both spontaneous and UV. Also look for data on growth of branched cells. (Twort)
2. More pertinent: 1),look for divided E further. 2) diagnose E,S sells by viscous media. 3) transfer intermediate chains for electron microscopy 4) clean up serotypes of co-segregants-- collect more? 5) For 4 and others need to complete review of data and write up.

3.TODAY: Clean up what is accumulated to look at and photograph.

Start new preps. of 93--x w/wo TZ. Use for divided clones and for Z correlation.

(Sat 5/7/55- Sun 5/8/55----)

Use TZ stained prepn. 5/6. 12n7 Checked first with 1237A1+ for swarm motility. In this series, used 2% methocel 400, diluted c. 1/10 with penassay.

a) use methocel for trap; b) isolate initials in broth trap, then trANSFER to mcl.

The latter was found ineffective (probably still too stiff); By 4 PM, had isolated 13 cells still sluggishly motile in mcl trap, and 7 addl. which were at a distance from reservoir but not now motile. swarm cells were sluggishly motile in this methocel conc., about 50-70% were directly inhibited. This oln. probably wets glass more effectively, at any rate it tends to spread, and a few of the motiles below may be contaminants from 1237A1+/

The motile residuals above were planted in individual drops of broth for class. as Sw. or E cells.

N8: found, in first group: 6 swarms, 3 E, 2 ng, 2 E.
second 4 E 1 ng 2 E

Total 6 S 7E 3ng 4 E

which demonstrates strong selection against E cells

Detailed counts:

	growth	motiles
1.	4+	9
2	3 + sw	swarm, 50%?
3	4+	2
4	ng	
5	4+	12 (from Z cell, but Z nf)
6	4+ trap 3+	0,1
7	sn, 1 mot cell	
8	20 sw	16, sev. shakes, prob. sw
9	sw sw	
10	sw sw	
11	like 8 sw	
12	500	12
13	200 sw sw	
21	4+	4
22	ng	
23	4+	18
24	4+	24
25	4+	2
26	4+	15
27	4+	16

(1o4)

The occasion was also used to plant about 25 single motiles (removed before test below--perhaps should have been left in it) for ~~opn~~ coordinance on immediate and later motility of dividing chain cell. About 12 usable cases no discrepancies, some to one or two later divisions. As none gave two motiles, pres, none of these were E . Of remainder, most gave two app. nm at this division-- it may be possible to reexamine these drops tomorrow. What is significance of this crisis in termination? Is is growth in fresh medium? (May still need a good exhausted medium to keep cell size small.)

P8 These were then used in tests for residual motility in mcl. Unf., 1,5 were washed in 5½% mcl 15 (calc. visc 200) which proved also to inh. swarms. Further tests were then made with mcl 400, 1.8% and 1% (1:1 penassay), the latter being adopted as it permits almost full motility of motile swarms(from above). (This may be too fluid for accurate discrimination against E, as will be seen). From E: 12,23,24 26, 27, ~~altogether~~ cells reisolated which remained motile were planted for further classification → none proved definite E cells. See further below.

Until this is worked out against misfutter predgries!

—x SW 666 initials in methocel and Z chains

b a serum.

DATE: May 9, 1955 REF:

Prestained pwepn. used. (st Z 9:45-12N; phage to 1:20, centr. and refr. 1:40) (also another preph. unstained = A.

Note immoderate spreading out of methocel droplets. isolate initials in 1% methocel 400 (1:1 2%: penassay).

A. isol. from unstained, plant out in droplets individually.

B. isol. Z-stained initials. to c. 3PM, some fresh isol. C to 4:30 me t in single drops on initial cg. first, transfer latter as families to isolation cg . Ditto for A— plant out descendants.

D. 5PM B above, in broth traps: pick c. 4000 initials (somewhat late now for tests) in serums.

(Klein visited 5/10-11.)

D: Almost all initials are inhibited in a or b serum, though cells may continue to spin for a few minutes. 7 cells did persist in b, planted out. 3 proved viable swarms. isolate as 1259 D1-3. See DCG for results of platings(after picking to broth)in MGA. 2 persisters in a, but neither viable.

E. same as B-D but not sublined

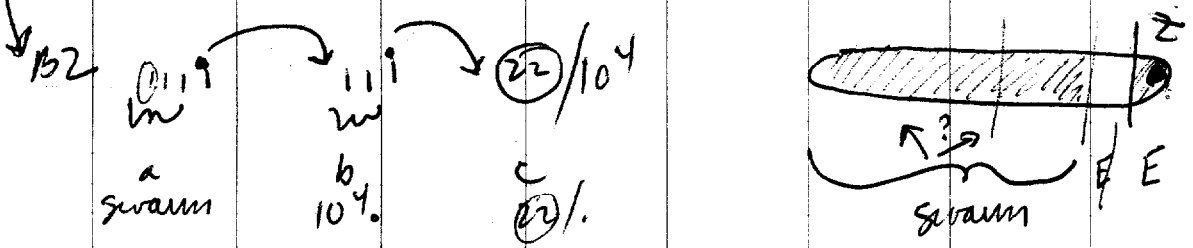
A: Held to 5/11 for examin, and may have partly diminished therefore.

13 clones 2? E clones. 1% Methocel probably too thin..

B: Most isolates grew out; had been separated once or twice at n_2-n_4. However, of
C: 38 isolations, 3 ng; 5 swarms; only 4E, none interesting except:
E-clones were reexamined for content. In B2, sib to swarms had 22 motiles, transferred to/238/ E2,3; 4; 5(sw). The motiles in E2 tested, all gave rise to inviable or E clones & therefore certainly not sw. cells/

not sublined

6% originally looked as if only c. 100+/10^4 but these later proved to be swarms. The clone was not recovered (owing to drying out) to verify originally low assay.



Otherwise, detailed numbers of intermediates were not recorded.
F+ 5/10 interm motiles tested in a serum: at least (28) from 5 clones were immbo. but 2/4 from B15 were not! However, two tested swarms were inhibited; specificity of serum should be rechecked.

Also saved 1259B1 (= b8). Swarm- test purity by plating
B2a,B (= c5 z cell removed at n_5 = nonmot,b) (not not certain record)

5/17

DCG found D1-3 all motile but with confusing clusters. B1: no definite swarms B2b "all clusters"; a pure non-motile. Will have to be rechecked on return (over)

E: 34 isolates planted w/o lineage afterward.

4E (9,11,15,16)
15E (1, 4,3,1,1,6,7,3,3,5,5,2,1,3,1,4,....)
5 Sw
5 ng.

Only conclusion: medium not adequately selective. Try 1½% methocel 400 (v.i.: 1260)

1259 summary to 5/16

5/12 Plated in MGA

5/13 Picked possible singles. Plates were incubated too short a time at 37°; D1 & D2 had singles, swarms, & clusters; D3, B1, & B2b singles & clusters only. Counts:

	Clusters & swarms	Singles	Singles picked
D1	51	3	2
D2	91	1	1
D3	59	5	4
B1	32	21	8
B2b	90	17	8

} these spotted on MGA

5/14 All "singles" picked 5/13 & spotted on MGA were motile (Spots had appearance of "clusters" rather than swarms).

Plated again: All original broths, + some of singles picked 5/13 (D1, 2; D2, 1; D3, 2; B1, 2; B2b, 2.) Incubated 3 hrs at 37°, overnight at 22°, then refrigerated until examined 5/16.

5/16 Results of 5/14 plating:

Original broths:

D1 Swarms, centered swarms, & col. ē "satellites"

D2 ~ D1, higher proportion of swarms.

D3 ~ D1.

B1 Trails, clusters, apparent singles; no swarms

B2a pure non-motile

B2b All clusters

Presumed Fla -:

D1(1) All clusters } no singles

D1(2) Clusters, swarms } no singles

D2 Clusters, swarms, no singles

D3(1) Clusters, swarms } no singles

D3(2) " " } no singles

B1(1) } Clusters, trails or satellites; possibly some singles;

B1(2) } no swarms.

Ni - 62 MAY 1 0 1955 *Best resume page*

- 1259B. ABCDE

In abc 3 swarms, 5E / 34 isolates

disappointing. No E cond. Z. But write out detail anyhow

B1 → 37+ → 13/104

B2 → 5/104, later 22

B10 → but only 1 drop seen.

B15 Rec. confused.

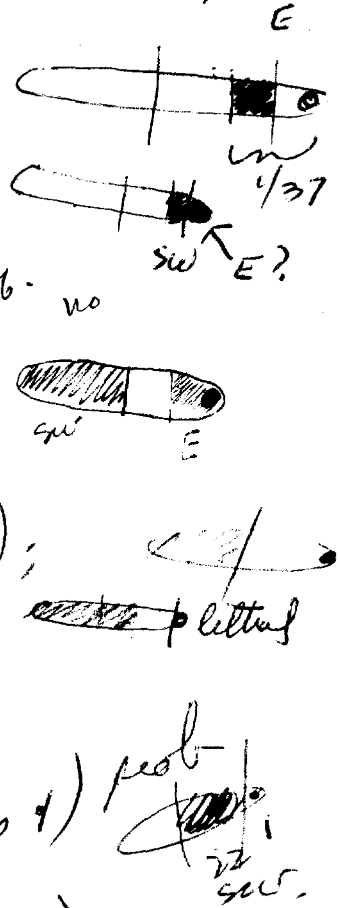
C1 → why 3 drops? (prob Lng.);

C2 → 1/9

C4 → But only 1 drop, + 100/104!

C5 1 inf → 1/22 → whence 3 drops? (3rd has 1) prob

C8 sw sw



(over)

C4 (+) many to 238 H2 } Hold for full analyses if needed.

Residue to F2, F3

C and B were evidently confused yesterday!!

MAY 1 0 1955

Do not save these swarms any to possible confusions.

But study closely B2 and C4.

B2 is swarm / 10%. / (5)/10%

(1)111/i → 11 10%

i (5)/10%

n_2 n_3 prob.

C4/is (1) → $\frac{>\times(100)}{10\%}$.

---

238 duid type pulsing

Partitions

May 13. New prepn., unstained. (probably usual, about 90-120mins.)

Fuse drops 2:30 Collect to 3:30. Cf 1259D motile.

No initial was nearly as active as 59D. Pick those that have moved the furthest, not necessarily v. active now. Estimated yield, 10% of broth yield.

Note : to compensate for spreading of methocel solution, use cg. that has been greased (human), then flamed. This works well, especially with larger drops, but smaller drops are too convex for best visualization. Intention was partly to look for early chains (E) in the methocel, but time did not allow and most isolates were made to broth directly/(A, B resp.) Lineages were separated at n_2-$_3$.

A: 1,2,3,6 ok. Partitions at n_1:

14+ :1 6:5 ng snakes. Later transferred entire clones to get fullest estimate of motiles.

Al came out +(14):6 Sepn at n_1 =

Bl-14,21-36. 4 ng. Mostly non E. Records show at first scanning:

2:4;1 14+ 2:1 3 7:20 0;4;1 1;3;d 3 2+ 2:1 1:0 5

sw;sw;sw;sw (1260B33 later DCG verified purity of each). 6:5 7:+

Underscores were rechecked (on ungreased slide!) and following definitobe values for splits on these:

1:20 8:20 2:2 4:12 3:2 7: 26 Therefore no equal splits.

General totals:

E 5
ng 4
sw 1
E

33

Little if any selection for E in 1½% methocel.400. Need 2% which probably totally stops many motile cells.

No new experiments after 5/14
Trip to NY 5/18 – 5/25. Resume lab work 5/3

→ x SW666
Methocel

MAY 31 1955
JUN 1 1955

Pupn 93 x SW666 (1:1) 10^{05} – 11^{45} (12^{30} in antifyt) SIC Af. & c. 4 PM.
c 4^{30}-5^{45} isol residual motiles. Est discrimination factor ca 1%.

A) Note: to prevent spread of methocel, e.g. are lightly greased with fingers (on one side); flamed; oil added. However, motile selection seemed most effective where there was appreciable wetting and spreading of the drop on the coverglass.
Zootes transferred to fresh pervassay drops c 6 PM, inc 30°

JUN 2 1955

Counts of (+) / 10^{3}-10^{4} : 4 25, 20, 13, 16, 11, 6, 2, 50 ; 3, 20, 18, 3, 20, 7, 11, 13, 10, swarm!
Σ : (6 E : 3 E̸ : 1 swarm ↓ 2 Inviable)

∴ with 2% methocel 400 there is effective discrimination. At this conc, Fla+ (1237A1+) was greatly slowed down (10x ?) but most cells did continue to move.

[Note — to this point considerable interruption in continuity of work was occasioned by ① trip to NY for ascites meeting ② breakdown of manipulator — hole in diaphragm, temporarily repaired]

∴ continue pedigree studies on preselected initials.

swarm: indirect plating of clone, in 1 ml, .01 ml gave 44 swarms / 265 singles
again note low ratio. Schedule completion!
(see photo — plate had been held at RT overnight, inc 2½ hours 37°, then RT 4 hours.

Mcl 400 2% solution.

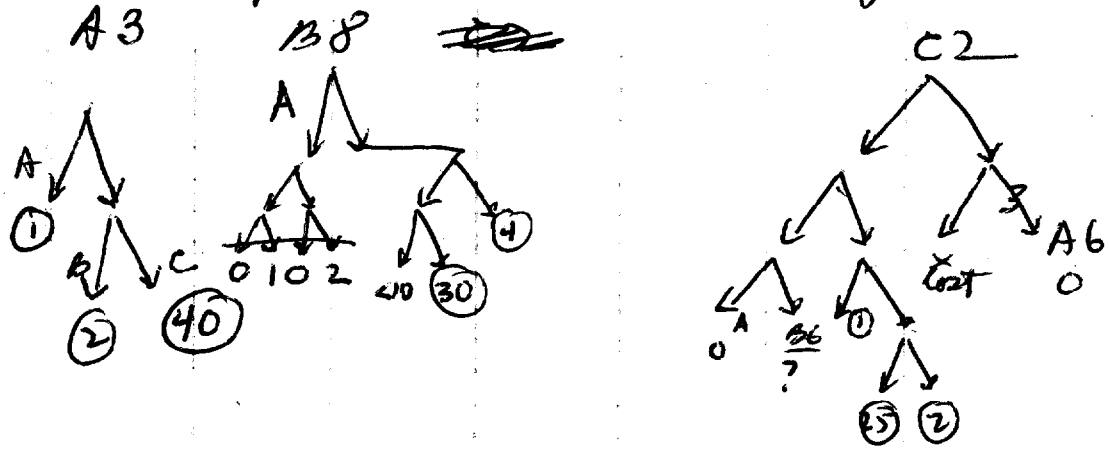
Pedigrees.

1262

JUN 2 1955

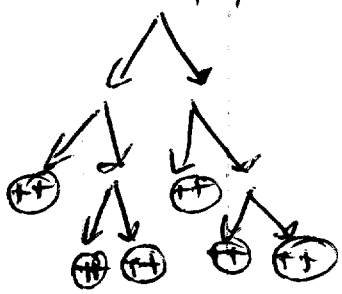
A). grow in penassay B. grow in Mcl. 1261 pups.
Plan to pure dups c. 12:10 PM.

No cells continuously motile like F1a+ clone were seen. Picked the most active. If continued, probably need to regulate the degree of wetting. Did pedigrees to 43-45 on 34 mutants transferred to broth.
Then pedigrees
P3, scan for E, ++, swarm. Found only 3 E clones.



∴ splits are 1:42 3:34 and 28?:0 (known 1:27)
2:25

One swarm clone C4, almost pure. DCC checked purity of each clone by plating.



Same! as 1262 C1-5
for H, check.

---

some plates were only 3E, 1S : 22 ++ and 10 ϕ (= lethal)
[11-0; 9-1; 2-2, 3, 4, 6, 7].,
43, 37+, 28
the experiment was quite unsuccessful.

Again review Salmonella data
to get paper out of the way.

July 13
1955

A) → Should first get general picture of experiments + what they were!

Write out 1138 B4

? → X SW666 4p+

Note diminished motility of large cells. Occ. early isol 1. → ①/10³...
(remarks on growth cycle) e.g. 1141 B4

7/55

1141 A4 v.p.

A5

B1

B3

B4 vp

A1, A3, B5, C1 n.q. (stayed motile)

C2

A4 (v.p.) Σ=33.

*① first suture!

note partition: 19:11
or 1:(19:11):1:1

But cannot use as G2 may be derived as from B4!

B4 If the latter, then

This datum is unreliable.

	Σ	a	b	c	d
3	1	1	5	—	
	2	6	6	11	
1	0	1	1	1	
	0	1	1	1	

	Σ	1	2	2	av 3.
A_2	1	2	2	19*	—
B_2 - D 15	18	6	16	16	29
- G2 15	1	20	27	27-29	37
				40	45
C_2	1 n.q.	2	2	2	2
B D 5	11	9	15	15	29
D_2	1	2	27		20
G2		16	23	33	47.
			23	24-26	34

subscript = point of this branch in the pedigree.

7/13/55

		Σ				
1142. ⑨	C 3	>50 5 tested	6	10	31	36
	D1	>100 10 tested	7	24	41	45
	C 4	10 2,1,3,1,0,0	3	10	—	— (no record)
1143	E 3	3	2	2	12	14
			3	12	—	—
	E 2	11	S_1 2	2	2	7
			S_1 2	2	3	3d
			S_2 3	10	16	23
			3	10	42	48
			S_7 5	11	27	31
			5	11	31	38
			5	11	14	—
			5	11	—	15
1144						

Leifson cultures.

1272
SEP 9 1955

all 6 cultures grow as well on both at 30° as at 37 except 205.

For preliminary comparisons, inoculate H1, H302, H32, H37 1:5 in broth + incubate 9AM —

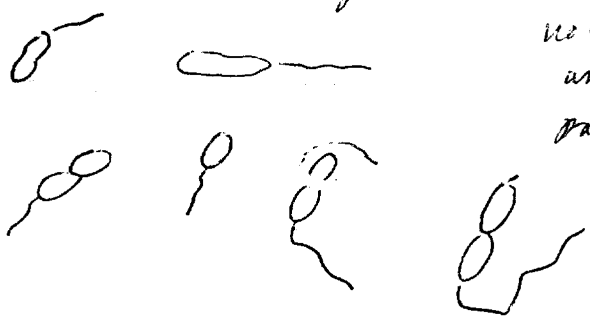
1272

SEP 8 1955

Leifson's slides See his letter figure pairs primarily

H1 A. 1200 x aprox.

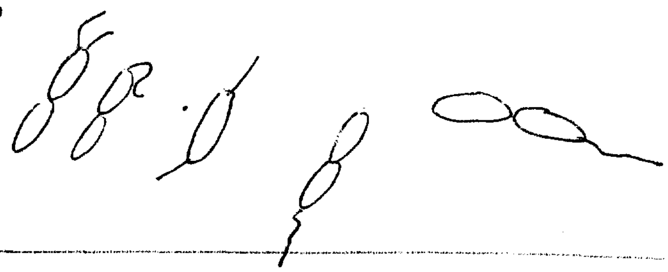
P. aeruginosa typ.



no clear antipolar pairs. sprouting?

polar monotr unip.

H 300



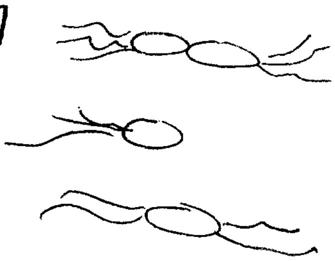
ditto
cells larger than above.

H32

vibrio?
multitrich, antipolar

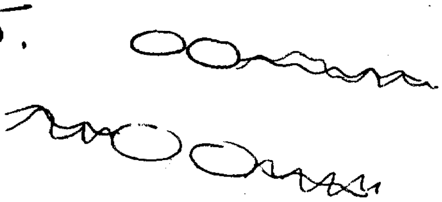
"alcaligenes"
(Lophobacter)

H37



large cells.

H205.



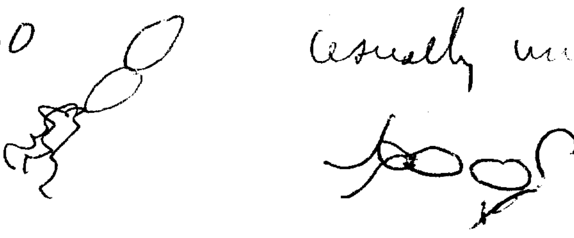
more usual

rare

prob. intermediate

polar multitrich
some bipolar!

H430



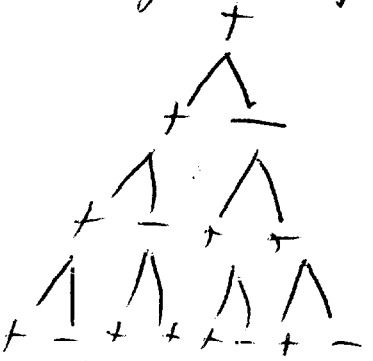
usually unipolar

(over)

All [242] protocols.

Conclusions

a) Pedigree possibly



b) No great regularity; some + < − / +

Some + < + / +

Could be studied further

It is just culture